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Functional Reconstitution of Olfactory Receptor for Analytical Application

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Olfactory Receptors; Enzymes; Proteins; Metabolism; Chemosensitive Devices; Protein Kinaces

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A model system was used to study the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium: Membrane from rat olfactory epithelial homogenates incorporated into planar bimolecular lipid membranes and patch-bilayers.

Data was obtained to support our hypothesis that the above described system in which we have demonstrated the presence of ion selective channels coupled with an activated enzyme cascade bears functional relationship to the initial chemoreceptive steps in olfaction.

Chemosensitivity is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

The kinetics of single channel events associated with the initial steps of olfaction

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have been studied.

Involvement of guanine nucleotide-binding regulatory proteins (G-proteins), of second messenger (c-AMP), and protein kinases have been studied using voltage and patch-clamp techniques together with application of pharmacological agents known to alter the metabolism or effects of the second messenger.

The study of the molecular mechanisms underlying the initial chemoreceptive events together with the development of stable reconstitution system should contribute toward the development of a practical analytical chemosensitive device.

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FUNCTIONAL RECONSTITUTION OF OLFACTORY RECEPTOR FOR ANALYTICAL APPLICATION.

Final report.

VITALY VODYANOY

October 15, 1989
U.S. ARMY RESEARCH OFFICE

GRANT NUMBER DAAL03-88-K-0077

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A. FOREWORD.

This proposal is entitled "Functional reconstitution of olfactory receptor for analytical application."

The basic objectives of the project were as the following:

1. Examination of the initial chemoreceptive events in the olfactory system of the rats, using the functional transfer of olfactory receptor membrane from homogenates of the olfactory epithelium into artificial systems.
2. Study of the physical and chemical properties of the chemosensitive ion channels in the reconstituted model system. Investigation of the functional relationship of these ion channels to the olfactory system.
3. Exploration of new approaches to increase the stability and sensitivity of the model chemosensitive system.

The key question of the project was: "How is odorant concentration translated into a functional electrical event in the olfactory receptor cell ?"

Significant progress has been made for most of the objectives of the project.

B. STATEMENT OF THE PROBLEM STUDIED.

We have used a model system to study the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium: Membrane from rat olfactory epithelial homogenates incorporated into planar bimolecular lipid membranes and patch-bilayers.

We have obtained data to support our hypothesis that the above described system in which we have demonstrated the presence of ion selective channels coupled with an activated enzyme cascade bears functional relationship to the initial chemoreceptive steps in olfaction.

Chemosensitivity is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

We have studied the kinetics of single channel events associated with the initial steps of olfaction.

Involvement of guanine nucleotide-binding regulatory proteins (G-proteins), of second messenger (c-AMP), and protein kinases have been studied using voltage and patch-clamp techniques together with application of pharmacological agents known to alter the metabolism or effects of the second messenger.

The study of the molecular mechanisms underlying the initial chemoreceptive events together with the development of stable reconstitution system should contribute toward the development of a practical analytical chemosensitive device.

C. SUMMARY OF THE MOST IMPORTANT RESULTS.

This work is concerned with the functional reconstitution of chemosensitive receptors from olfactory epithelium of the rat and also with molecular mechanisms of ion transport associated with olfaction.

We have used three different techniques to transfer the native membrane macromolecules into a model system: (1) Chemosensitive membrane fragments from olfactory cilia were incorporated into bimolecular lipid membranes of large surface area (1 mm^2); (2) Vesicles which contained chemosensitive membrane fragments were attached to the large planar bilayer, and conductance of the membrane system

was modified with ion carrier; and (3) The cilia membrane was functionally reconstituted in patch electrode membranes.

We have developed and used an interactive software capable of identifying single-channel transitions in the presence of substantial levels of noise and drift.

Clustering of single-channel openings was found in presence of cAMP and ATP. We suggest that cyclic gating scheme may result in correlation of successive dwell times, and the irreversible steps included in this cycle may require an energy supply to maintain the steady state.

We have demonstrated that cAMP mimicted the effect of odorant. The statistical analysis of our patch-clamp data suggested the multiple mode of cAMP action on the single channel activity: (a) directly, and (b) via protein kinase system. We hypothesize, that chemosensitivity of functionally reconstituted olfactory receptor is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

Functional transfer of chemosensitive monolayers, membranes and multilayers on to solid substrates can be proposed as a structural basis for development of membrane sensor.

Functional transfer of chemosensitive monolayers, membranes and multilayers on to solid substrates can be proposed as a structural basis for development of membrane sensor.

D. LIST OF PUBLICATIONS ACKNOWLEDGING THE ARO.

1. "Alamethicin-induced voltage-dependent capacitance in planar lipid bilayers". I.Vodyanoy, J.E.Hall and V.Vodyanoy, Biophys J., 53, 649-658, 1988.

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